

## Hybridization and Chloroplast Captures in *Asarum* sect. *Asiasarum* (Aristolochiaceae) Documented by Chloroplast DNA Sequences

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The phylogenetic relationships of *Asarum* sect. *Asiasarum* based on genetic variation in non-coding chloroplast DNA sequences were examined. A total of ca. 960 bp sequences of three non-coding regions, namely *trnL* (UAA) intron, *rps16* intron and *psbC-trnS* (UGA) intergenic region, were analyzed for 37 accessions from 13 taxa and one geographic race. In the maximum-parsimony (MP) tree, four types were recognized. Among the 13 taxa and one geographic race, 10 taxa were identified as belonging to a single type, while the remaining three taxa and one geographic race, *A. patens*, *A. sieboldii* f. *sieboldii*, *A. heterotropoides* var. *seoulense* (from Korea) and *A. versicolor*, belonged to two types. The types all corresponded to more than one ribotype recognized in previous of nrDNA, ITS sequences. The phylogenetic tree topologies were difficult to compare. Chloroplast polymorphisms within a taxon and incongruence of the phylogenetic tree topologies between chloroplast and ITS were considered to be caused by chloroplast capture following hybridization. Reticulate evolution in sect. *Asiasarum* was therefore inferred to be more extensive than implied from ITS sequence data. *Asarum maruyamae* was speculated as being of hybrid origin from crosses between *A. sieboldii* f. *sieboldii* and *A. mikuniense*.

Key words: Aristolochiaceae, *Asarum* sect. *Asiasarum*, chloroplast capture, molecular phylogeny, reticulate evolution

Species of *Asarum* L. sect. *Asiasarum* Araki (Aristolochiaceae) are long-lived rhizomatous herbs with a distribution in Japan, China, Korea, and eastern Russia (Maekawa 1936, Cheng & Yang 1983, Kelly 1998, Yamaki *et al.* 1996, Lee & Lee 2000). Section *Asiasarum*, comprising ten species and three varieties (Yamaji *et al.* 2007a, b, c), presents many taxonomic difficulties, as evidenced by the discrepancies in previous taxonomic treatments in

the limits of taxa and the rank at which some taxa should be recognized. The taxa have been recognized on the basis of more than one character. The discrepancies between systems are due to differences of opinion on which characters are the important ones. Taxa with intermediate character states are possibly of hybrid origin.

A previous molecular study on the internal transcribed spacer (ITS) of nuclear ribosomal DNA

regions revealed the phylogenetic relationships of the taxa within *Asarum* sect. *Asiasarum* and the extensive reticulate evolution in the group (Yamaji *et al.* 2007c). In the ITS sequences, eight taxa comprised a single ribotype while the other five taxa (*A. heterotropoides* F. Schmidt var. *heterotropoides*, var. *mandshuricum* (Maxim.) Kitag., var. *seoulense* (Nakai) Kitag., *A. misandrum* B. Oh & J. Kim, *A. versicolor* (Yamaki) Y. Lee) were composed of two ribotypes. Because all taxa in sect. *Asiasarum* are diploid ( $2n = 26$ ; Ono 1960, Sugawara 1981, Choi *et al.* 1998, Yamaji unpubl. data), the taxa containing two ribotypes were suspected as being of diploid hybrid origin and to have retained their parental ribotypes (Yamaji *et al.* 2007c). The discrepancies between the taxonomic systems therefore appeared to be caused in part by extensive reticulate evolution.

Problems in understanding the phylogeny of sect. *Asiasarum*, however, have not been settled. One of the unresolved putative hybrids, *Asarum maruyamae* Yamaji & Ter. Nakam., is similar to *A. sieboldii* Miq. f. *sieboldii* in floral color pattern and chemical components, whereas it is also similar to *A. mikuniense* Yamaji & Ter. Nakam. in floral shape and pollen characteristics (Nakamura 1986, Yamaji *et al.* 2007a, b). In the ITS sequences, *A. maruyamae* does not exhibit a combination of ribotypes, and forms a monophyletic clade with *A. mikuniense* and *A. tohokuense* Yamaji & Ter. Nakam., but not with *A. sieboldii* f. *sieboldii* (Yamaji *et al.* 2007c). It is possible that the parental ITS sequences of *A. maruyamae* have been fixed to those of *A. tohokuense* or *A. mikuniense* by concerted evolution, which is mechanism to homogenize polymorphisms in tandemly repeated nuclear ribosomal DNA in the same or different loci within a lineage (Aguilar *et al.* 1999).

For further examination, analyses of the other genetic markers inherited independent of ITS inheritance would be useful. Inconsistencies between cytoplasmic (e.g. chloroplast) DNA phylogeny and nuclear DNA (e.g. ITS) phylogeny have been doc-

umented in many plant groups and are considered to be the result of chloroplast capture through introgression (Rieseberg & Soltis 1991, Rieseberg *et al.* 1996, Wolfe & Elisens 1995, Tsitrone *et al.* 2003).

In this study, we examined the non-coding regions of chloroplast DNA sequences to add to the phylogenetic information of *Asarum* sect. *Asiasarum* and to resolve the status of putative hybrids and chloroplast captures to compare with the ITS phylogenetic tree.

## Materials and Methods

### Plant materials

Thirty-seven accessions of 13 taxa and one geographic race of sect. *Asiasarum* were sampled in this study (Table 1, Fig. 1). All of the samples were already examined for their ITS sequences in a previous study (Yamaji *et al.* 2007c). We divided *Asarum heterotropoides* var. *seoulense* into two geographic groups, based on source (Korea or China) because they differ in floral characteristics. *Asarum caulescens* Maxim. (from sect. *Asarum*), *A. epigynum* Hayata (from sect. *Geotaenium* (F. Maek.) L. Kelly), and *A. muramatsui* Makino (from sect. *Heterotropa* (Morr. & Decne.) A. Braun), were selected as outgroups because they are considered to be closely related to sect. *Asiasarum* (Kelly 1998).

### DNA isolation and amplification

Total genomic DNA was isolated from 200 to 300 µg of fresh leaf tissue or herbarium specimens by the modified 2×CTAB procedure of Doyle and Doyle (1987). The isolated DNA was resuspended in 100 to 200 µL TE. The regions to investigate were selected by a preliminary examination and then three informative regions were selected: *trnL* (UAA) intron, *rps16* intron, and *psbC-trnS* (UGA) intergenic. PCR amplifications were achieved using universal primers, c and d for *trnL* (UAA) intron (Taberlet *et al.* 1991), rps16/2F and rps16/2R for

TABLE 1. Locality, voucher specimens, and accession numbers of populations of taxa examined for chloroplast DNA sequences. All samples were also examined for ITS sequences. Sample names are consistent with the examination. Voucher specimens are deposited in TUS or THS.

Sample name	Source	Voucher	<i>trnL</i> (UAA) intron	Accession No. <i>rps16</i> intron	<i>psbC-trnS</i> (UGA)
sect. <i>Asiasarum</i>					
<i>A. heterotropoides</i> var. <i>heterotropoides</i> -4	Japan, Hokkaido, Kamikawa, Furano-shi	<i>H. Yamaji 7126</i> (TUS)	AB299859	AB299896	AB299933
<i>A. heterotropoides</i> var. <i>heterotropoides</i> -13	Japan, Hokkaido, Hidaka, Samani-cho	<i>H. Yamaji 7105</i> (TUS)	AB299860	AB299897	AB299934
<i>A. heterotropoides</i> var. <i>heterotropoides</i> -25	Japan, Hokkaido, Oshima, Minamikayabe-cho	<i>H. Yamaji 7101</i> (TUS)	AB299861	AB299898	AB299935
<i>A. heterotropoides</i> var. <i>heterotropoides</i> -27	Japan, Tohoku, Aomori, Kawauchi-cho	<i>H. Yamaji 7023</i> (TUS)	AB299862	AB299899	AB299936
<i>A. heterotropoides</i> var. <i>heterotropoides</i> -31	Japan, Tohoku, Miyagi, Kurikoma-cho	<i>H. Yamaji 6120</i> (TUS)	AB299863	AB299900	AB299937
<i>A. heterotropoides</i> var. <i>mandshuricum</i> -12	China, Liaoning, Benxu Manzu Zizhixian	<i>THS 76900</i> (THS)	AB299864	AB299901	AB299938
<i>A. heterotropoides</i> var. <i>mandshuricum</i> -13	Korea, Kangwon-do, Myogju-gun	<i>H. Yamaji 8180</i> (TUS)	AB299865	AB299902	AB299939
<i>A. heterotropoides</i> var. <i>seoulense</i> KOREA-2	Korea, Kyonggi-do, Kanghwa-gun	<i>H. Yamaji 8155</i> (TUS)	AB299866	AB299904	AB299940
<i>A. heterotropoides</i> var. <i>seoulense</i> KOREA-3	Korea, Kyonggi-do, Kanghwa-gun	<i>H. Yamaji 8150</i> (TUS)	AB299867	AB299905	AB299941
<i>A. heterotropoides</i> var. <i>seoulense</i> CHINA-1	Russia, Vladivostok	<i>THS 76905</i> (THS)	AB299868	AB299906	AB299942
<i>A. heterotropoides</i> var. <i>seoulense</i> CHINA-11	China, Liaoning, Benxu Manzu Zizhixian	<i>THS 76902</i> (THS)	AB299869	AB299907	AB299943
<i>A. heterotropoides</i> var. <i>seoulense</i> CHINA-12	China, Liaoning, Benxu Manzu Zizhixian	<i>THS 44829</i> (THS)	AB299870	AB299908	AB299944
<i>A. misandrum</i> -2	Japan, Kyushu, Kumamoto, Aso-cho	<i>H. Yamaji 6215</i> (TUS)	AB299871	AB299909	AB299945
<i>A. mikuniense</i> -1	Japan, Kanto, Gunma, Niiharu-mura	<i>H. Yamaji 6235</i> (TUS)	AB299872	AB299910	AB299946
<i>A. mikuniense</i> -2	Japan, Chubu, Nagano, Sakae-mura	<i>H. Yamaji 6220</i> (TUS)	AB299873	AB299911	AB299947
<i>A. mikuniense</i> -3	Japan, Chubu, Niigata, Myoko-mura	<i>H. Yamaji 6234</i> (TUS)	AB299874	AB299912	AB299948
<i>A. mariyamae</i> -1	Japan, Chugoku, Shimane, Nita-cho	<i>H. Yamaji 7001</i> (TUS)	AB299875	AB299913	AB299949
<i>A. mariyamae</i> -2	Japan, Chugoku, Shimane, Nita-cho	<i>H. Yamaji 7002</i> (TUS)	AB299876	AB299914	AB299950
<i>A. tohokuense</i> -1	Japan, Tohoku, Aomori, Nakatsugaru-gun	<i>H. Yamaji 7021</i> (TUS)	AB299877	AB299915	AB299951
<i>A. tohokuense</i> -2	Japan, Tohoku, Fukushima, Kori-cho	<i>H. Yamaji 6061</i> (TUS)	AB299878	AB299916	AB299952
<i>A. tohokuense</i> -4	Japan, Chubu, Nagano, Otari-mura	<i>H. Yamaji 6045</i> (TUS)	AB299879	AB299917	AB299953
<i>A. versicolor</i> -1	Korea, Kangwon-do, Wonju-gun	<i>H. Yamaji 8120</i> (TUS)	AB299880	AB299918	AB299954
<i>A. versicolor</i> -2	Korea, Kangwon-do, Inje-gun	<i>H. Yamaji 8175</i> (TUS)	AB299881	AB299919	AB299955
<i>A. versicolor</i> -5	Korea, Ch'ungch'ongbuk-do, Mt. Songnisan	<i>H. Yamaji 8100</i> (TUS)	AB299882	AB299920	AB299956
<i>A. sieboldii</i> f. <i>sieboldii</i> -2	Japan, Chubu, Nagano, Iida-shi	<i>H. Yamaji 6051</i> (TUS)	AB299883	AB299903	AB299957
<i>A. sieboldii</i> f. <i>sieboldii</i> -6	Korea, Daegu City	<i>H. Yamaji 8090</i> (TUS)	AB299884	AB299921	AB299958
<i>A. sieboldii</i> f. <i>sieboldii</i> -9	China, Hubei, Shennongjia Linqu	<i>THS 67577</i> (THS)	AB299885	AB299922	AB299959
<i>A. sieboldii</i> f. <i>maculatum</i> -1	Korea, Chollabuk-do, Puan-gun	<i>H. Yamaji 8070</i> (TUS)	AB299886	AB299923	AB299960
<i>A. sieboldii</i> f. <i>maculatum</i> -4	Korea, Chollabuk-do, Sogwip'o-shi	<i>H. Yamaji 8140</i> (TUS)	AB299887	AB299924	AB299961
<i>A. sieboldii</i> f. <i>cornutum</i> -1	Korea, Kangwon-do, Inje-gun	<i>H. Yamaji 8170</i> (TUS)	AB299888	AB299925	AB299962
<i>A. dimidiatum</i> -2	Japan, Shikoku, Tokushima, Waki-machi	<i>H. Yamaji 8002</i> (TUS)	AB299889	AB299926	AB299963
<i>A. dimidiatum</i> -3	Japan, Kyushu, Miyazaki, Kitakawa-cho	<i>H. Yamaji 8006</i> (TUS)	AB299890	AB299927	AB299964
<i>A. patens</i> -2	Korea, Kyongsangnam-do, Kumi-shi	<i>H. Yamaji 8050</i> (TUS)	AB299891	AB299928	AB299965
<i>A. patens</i> -5	Korea, Chollanam-do, Kurye-gun	<i>H. Yamaji 8078</i> (TUS)	AB299892	AB299929	AB299966
outgroups					
<i>A. muramatsui</i> Makino (subgenus <i>Heterotropa</i> , sect. <i>Heterotropa</i> )		<i>THS 70571</i> (THS)	AB299893	AB299930	AB299967
<i>A. caulescens</i> Maxim. (subgenus <i>Asarum</i> , sect. <i>Asarum</i> )		<i>THS 79144</i> (THS)	AB299894	AB299931	AB299968
<i>A. epigynum</i> Hayata (subgenus <i>Asarum</i> , sect. <i>Geotaenium</i> )		<i>THS 79143</i> (THS)	AB299895	AB299932	AB299969

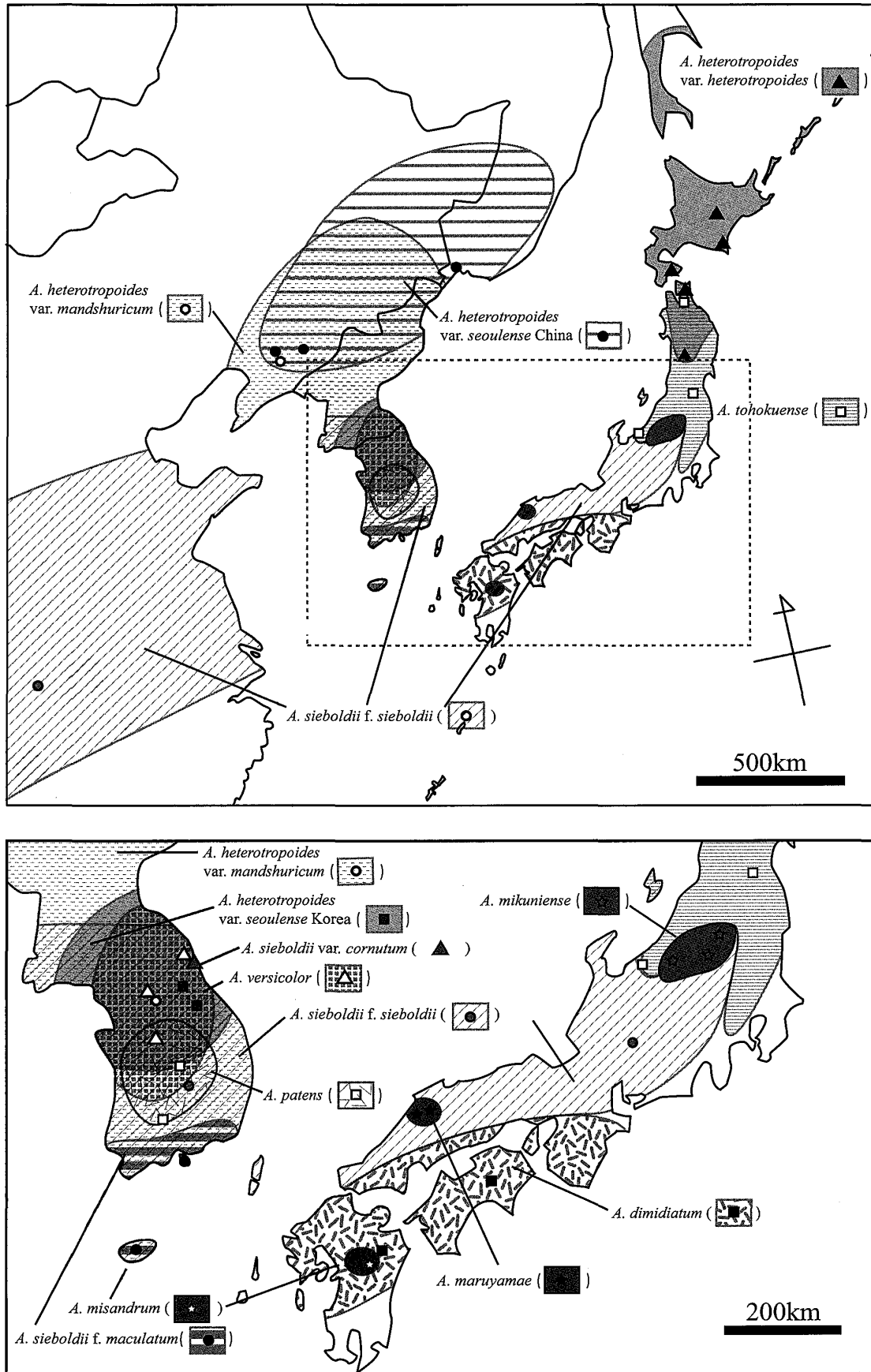


FIG. 1. The distribution of each taxon and geographic race, and the localities of samples examined in this study.

*rps16* intron, *psbC-tmS/F* and *psbC-tmS/R* for *psbC-trnS* (UGA) intergenic sequence (Nishizawa & Watano 2000). We used the following thermocycle protocol: 1 cycle of 2 min at 94° C, 30 cycles of 1.5 min at 94° C, 2 min at 48° C, 3 min at 72° C, and 1 cycle of 15 min at 72° C. The PCR reaction mixture consisted of 10×Ex-Taq Buffer (TaKaRa) 5 µL, dNTP mix (TaKaRa) 4 µL, forward primer (10 pmol/µL) 1 µL, reverse primer (10 pmol/µL) 1 µL, Ex-Taq (TaKaRa) 0.25 µL, DMSO 5 µL, D.D.W. 32.5 µL, and template DNA 1.25 µL. PCR products were separated from other byproducts using 1% TAE agarose gel electrophoreses. The desired bands were cut out and purified using GFX<sup>TM</sup> PCR DNA and Gel Band Purification Kit (Amersham biotech).

#### Sequencing the PCR products

We sequenced the purified PCR products using the BigDye Terminator ver. 3.1 Cycle Sequencing Kit and Model 3100 automated sequencer (Applied Biosystems), following the manufacturer's instructions. We used the same primers as those used for amplification. The sequences were aligned manually.

#### Phylogenetic analysis

Phylogenetic relationships were analyzed using the maximum-parsimony (MP) method (Swofford 2004). For all the analyses, we used PAUP 4.0b10 (Swofford 2004). All characters were weighted equally. Indels were binary-coded. The MP analyses were conducted through a heuristic search with a TBR branch-swapping option. In each analysis, 100 rounds of random sequence additions were performed to identify multiple islands of equally most parsimonious trees (Maddison 1991). Bootstrap analysis (Felsenstein 1985) with 1000 replications and 100 rounds of random sequence additions for each replication was performed using the same program.

## Results

The *trnL* (UAA) intron, *rps16* intron, and *psbC-trnS* (UGA) intergenic region from species in sect. *Asiasarum* were 445-446 bp, 207 bp and 299-306 bp in length, respectively. Among them, there were four nucleotide substitutions and three indels were observed (Fig. 2). Among the four nucleotide substitutions, two were autapomorphic; the other two were phylogenetically informative. Among the three indels, two were poly A and poly T tracts, and they were excluded from the phylogenetic analyses because of their possible parallel evolution caused by their high mutation rates (Provan *et al.* 2001).

In the MP analyses of the cpDNA sequences, we obtained only one most-parsimonious tree with 39 steps. The consistency index (CI) excluding uninformative characters was 1.00 (Fig. 3). We have defined four types (type A-D) within sect. *Asiasarum* (Figs. 2, 3). Type A was placed in the basal assemblage of sect. *Asiasarum*. Type B, type C+D, and type D formed monophyletic clades, respectively, and type C was placed in basal assemblage of the monophyletic clade C+D. Types A, B, C, and D included 4, 7, 4, and 3 taxa, respectively. Among the 13 taxa and one geographic race, 10 taxa were of a single type. The remaining three taxa and one geographic race were of more than one type; *Asarum patens* (Yamaki) Y. Lee was of type A and B while *A. sieboldii* f. *sieboldii*, *A. heterotropoides* var. *seoulense* KOREA and *A. versicolor* were recognized as types B and C. In comparison with the MP consensus tree of the ITS sequences (Yamaji *et al.* 2007c; Fig. 3), correspondence between the four types and the eight ITS ribotypes were examined. All types corresponded to more than one ribotype. Type A corresponded with ribotypes P and M because in samples with type A haplotype, *A. patens* contained ribotype P. *Asarum heterotropoides* var. *seoulense* CHINA, var. *mandshuricum*, *A. misandrum* contained ribotypes P and

Type	samples	region position	trnL intron			rps16 intron			psbC-trnS (UGA) intergenic		
			1	8	2	1	3	3	1	1	1
outgroups	<i>A. muramatsui</i>										
	<i>A. caulescens</i>										
	<i>A. epigynum</i>										
Type A	<i>A. patens</i> -5										
	<i>A. heterotropoides</i> var. <i>seoulense</i> CHINA-1										
	<i>A. heterotropoides</i> var. <i>seoulense</i> CHINA-11										
	<i>A. heterotropoides</i> var. <i>seoulense</i> CHINA-12										
	<i>A. heterotropoides</i> var. <i>mandshuricum</i> -12										
	<i>A. heterotropoides</i> var. <i>mandshuricum</i> -13										
	<i>A. misandrum</i> -2										
Type B	<i>A. patens</i> -2										
	<i>A. sieboldii</i> f. <i>sieboldii</i> -2										
	<i>A. sieboldii</i> f. <i>sieboldii</i> -6										
	<i>A. sieboldii</i> f. <i>maculatum</i> -1										
	<i>A. sieboldii</i> f. <i>maculatum</i> -4										
	<i>A. dimidiatum</i> -2										
	<i>A. dimidiatum</i> -3										
	<i>A. heterotropoides</i> var. <i>seoulense</i> KOREA-2										
	<i>A. maruyamae</i> -1										
	<i>A. maruyamae</i> -2										
Type C	<i>A. versicolor</i> -2										
	<i>A. sieboldii</i> var. <i>cornutum</i> -1										
	<i>A. sieboldii</i> f. <i>sieboldii</i> -9										
	<i>A. heterotropoides</i> var. <i>seoulense</i> KOREA-3										
	<i>A. versicolor</i> -1										
Type D	<i>A. versicolor</i> -5										
	<i>A. tohokuense</i> -1										
	<i>A. tohokuense</i> -2										
	<i>A. tohokuense</i> -4										
	<i>A. mikuniense</i> -1										
	<i>A. mikuniense</i> -2										
	<i>A. mikuniense</i> -3										
	<i>A. heterotropoides</i> var. <i>heterotropoides</i> -4										
	<i>A. heterotropoides</i> var. <i>heterotropoides</i> -13										
	<i>A. heterotropoides</i> var. <i>heterotropoides</i> -25										
	<i>A. heterotropoides</i> var. <i>heterotropoides</i> -27										
	<i>A. heterotropoides</i> var. <i>heterotropoides</i> -31										

Fig. 2. Variation of *Asarum* sect. *Asiasarum* in trnL intron, rps16 intron, and psbC-trnS (UGA) intergenic partial sequences. Samples are classified into four types by their variable states except poly A and poly T tracts. Taxa recognized in more than one type are shaded. The numbers indicate the relative positions determined from the result of multiple alignments of all samples.

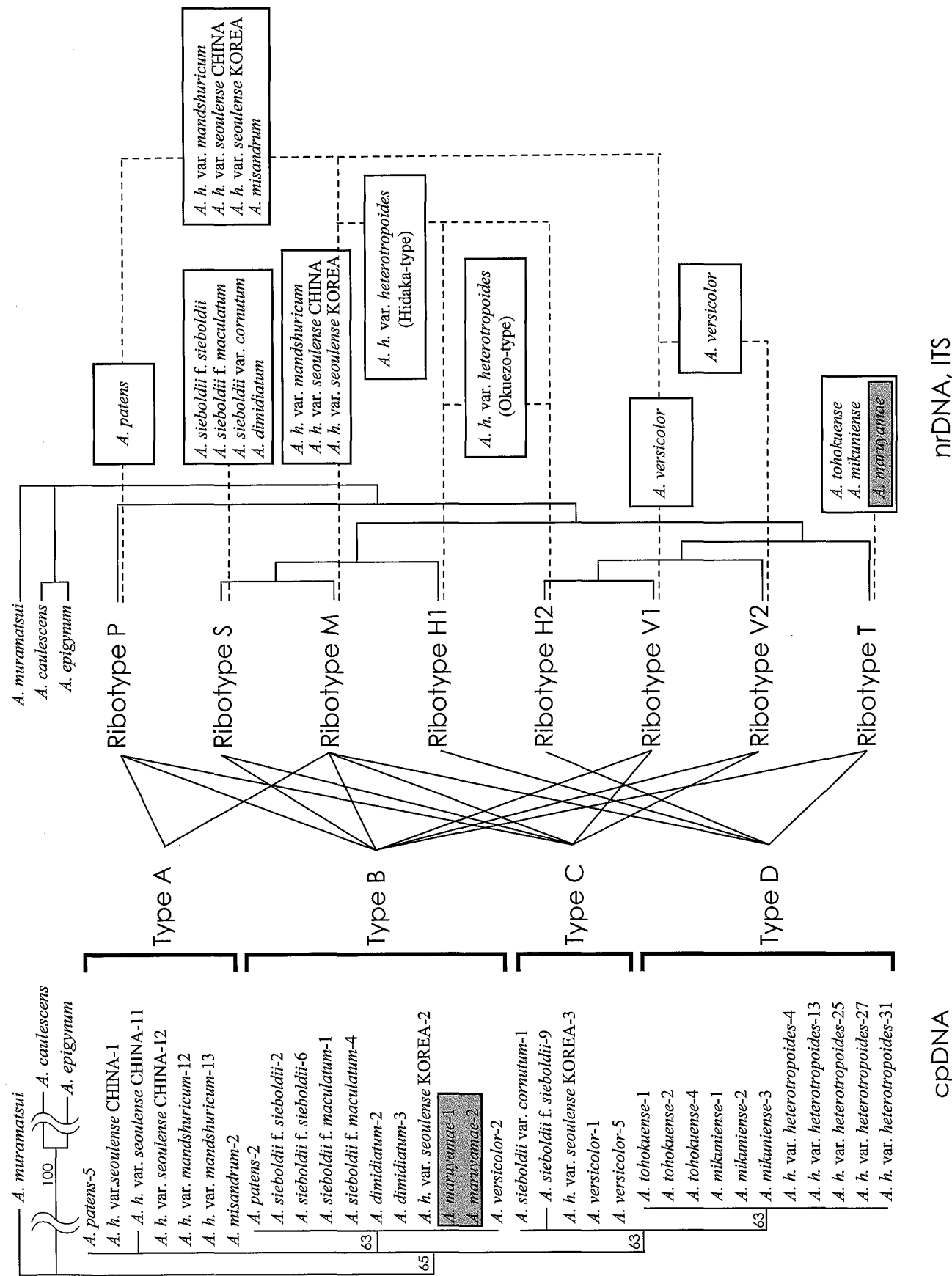


Fig. 3. Comparison between MP trees estimated from cpDNA and ITS sequences in *Asarum* sect. *Asiasarum*. MP tree of cpDNA was estimated from *trnL* intron, *rps16* intron, and *psbC-trnS* (UGA) intergenic region partial sequences. Only one parsimonious tree of 39 steps (CI=1.00; RI=1.00). Bootstrap values are given above branches of clades. ITS tree shown is summarized from results of Yamaji *et al.* (2007c). Boxes and dashed lines on right side of ITS tree indicate taxa containing each ribotype and reticulate relationship of sect. *Asiasarum*. Lines between clades in cpDNA tree and ribotypes in ITS tree indicate correspondence estimated from taxa composition. Shaded boxes indicate phylogenetic position of *A. maruyamae*.

M. Similarly, type B corresponded to ribotypes P, S, M, V1, V2, and T, type C corresponded to ribotypes P, S, M, V1, and V2, and type D corresponded to ribotypes M, H1, H2, and T.

*Asarum maruyamae* is a putative hybrid within sect. *Asiasarum*. Its phylogenetic position was remarkably different between cpDNA and ITS trees. It shared the type B haplotype with *A. sieboldii* f. *sieboldii*, although *A. sieboldii* f. *sieboldii* formed a monophyletic clade with *A. tohokuense* and *A. mikuniense* in the ITS tree. Although *A. heterotropoides* var. *heterotropoides* showed comparatively large variation in its ITS sequences, all five samples of this taxon had the same type D haplotype.

## Discussion

Our results showed intraspecific polymorphism in chloroplast sequences in 4 of 13 taxa and one geographic race: *Asarum patens*, *A. sieboldii* f. *sieboldii*, *A. heterotropoides* var. *seoulense* KOREA, and *A. versicolor*. In the MP tree of cpDNA sequences these taxa were scattered across more than one type. Moreover, the tree topologies of the cpDNA and ITS sequences cannot be simply compared because all the types in cpDNA phylogeny corresponded with more than one ribotype.

In many plants, chloroplast polymorphisms across two or more taxa and incongruence of phylogenetic reticulations between cpDNA and ITS trees are considered to be caused by chloroplast capture following hybridization (Tsitrone *et al.* 2003). In sect. *Asiasarum*, chloroplast capture supposedly is extensive. The chloroplast polymorphisms appear to be related to the pattern of distribution of the taxa (Fig. 1). The taxa with more than one haplotype are sympatrically distributed on the Korean Peninsula (Yamaki *et al.* 1996, Yamaji *et al.* 2007c); *A. patens*, *A. heterotropoides* var. *seoulense* KOREA, and *A. versicolor* are endemic to the Korean Peninsula, whereas *A. sieboldii* f. *sieboldii*

is widely distributed on the Korean Peninsula, in western Honshu, Japan, and in central and eastern China, but broad sympatry with other taxa occurs only on the Korean Peninsula. By contrast, taxa with a single haplotype were allopatric or parapatric, mainly in the Japanese Archipelago and northeastern China.

Overlapping distributions can result in introgressive hybridization between species of sect. *Asiasarum*. ITS polymorphisms presumably caused by introgressive hybridization were also recognized among the taxa distributed in these areas, although the ITS sequences were rather consistent with the taxonomic boundaries (Yamaji *et al.* 2007c). The ITS sequences are considered to be stabilized by backcrossing to one of the parental species and by consequent concerted evolution (Aguilar *et al.* 1999), whereas chloroplast capture might sometimes reflect past hybridization (Wolfe & Elisens 1995).

All taxa except *Asarum misandrum* with a type A haplotype occur on the Eurasian Continent. *Asarum misandrum* has obviously spread from southern Korea rather recently because of its limited distribution in Japan and because it has the same ITS sequence as *A. heterotropoides* var. *mandshuricum* and var. *seoulense* (Yamaji *et al.* 2007c). Since the type A haplotypes are placed in a basal position, sect. *Asiasarum* is speculated to have originated on the Eurasian continent and spread to the Japanese Archipelago. The conclusions from observation of the ITS sequences coincide with those (Yamaji *et al.* 2007c).

One of the putative hybrids, *Asarum maruyamae*, is distributed in a restricted area of Shimane Prefecture, western Honshu (Yamaji *et al.* 2007b). The phylogenetic position of these plants based on the cpDNA obviously differs from the position inferred from the ITS tree. These putative hybrids contain the same cpDNA haplotype as *A. sieboldii* f. *sieboldii*, *A. sieboldii* f. *maculatum* (Nakai) Yamaji, and *A. dimidiatum* F. Maek., but not *A. tohokuense*



and *A. mikuniense*. Because chloroplast genomes are maternally inherited in a majority of the angiosperms (Mogensen 1996), *A. maruyamae* is therefore considered to be a hybrid derived from crosses between *A. sieboldii* f. *sieboldii* as the maternal parent and *A. mikuniense* or *A. tohokuense* as the paternal parent.

The putative paternal parents, *Asarum tohokuense* and *A. mikuniense*, from eastern and northern Honshu, and high altitude areas of northern Kanto, respectively, do not occur sympatrically with *A. maruyamae*. The putative maternal parent, *A. sieboldii* f. *sieboldii*, which is widely distributed in central and western Honshu, Korea, and central and western China, does occur with *A. maruyamae* (Fig. 1; Yamaji *et al.* 2007b). *Asarum mikuniense* is rather plausible as the maternal parent because the narrow calyx throat is common in *A. maruyamae* (Yamaji *et al.* 2007a, b). Since the distribution of *A. maruyamae* does not overlap with *A. tohokuense* and *A. mikuniense*, which are rather boreal species, *A. maruyamae* possibly originated from a relic population of the paternal parent. *Asarum tohokuense* and *A. mikuniense* may have migrated during glacial cycles, as suggested by patterns in some alpine and cool temperate plants (Fujii *et al.* 2002, Fujii & Senni 2006, Ikeda & Setoguchi 2006).

In contrast, hybridization events involving *Asarum heterotropoides* var. *heterotropoides* were inferred from both morphological characteristics (Yamaji *et al.* 2006) and from the polymorphisms of the ITS sequences (Yamaji *et al.* 2007c). In the ITS sequences, more than one geographic race was recognizable. They are supposedly derived from different hybridization events. The 'Okuezo-type' race (*A. heterotropoides* var. *heterotropoides*-4, 25, 27, 31) has ribotypes H1 and H2, and is presumed to be a hybrid involving a previously homogenized species, whereas the 'Hidaka-type' race (*A. heterotropoides* var. *heterotropoides*-13) has ribotypes M and H1 or H2 and is presumed to be a hybrid

between the 'Okuezo-type' race and *A. heterotropoides* var. *seoulense*. The sample belonging to the 'Hidaka-type' had the same haplotype D, which was not assignable to *A. heterotropoides* var. *seoulense* (KOREA and CHINA) but to *A. tohokuense* and *A. mikuniense*. The chloroplast genomes of *A. heterotropoides* var. *heterotropoides* were possibly introduced from *A. tohokuense* or *A. mikuniense*. To clarify past chloroplast capture, it would be useful to determine whether these species have differentiated or not in their cpDNA by investigating an extended region of the cpDNA.

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